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=> s cross linking and biomaterial and precursor pharmaceutical
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SEARCH ENDED BY USER
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=> s cross linking and biomaterial and precursor and pharmaceutical
L1          12 CROSS LINKING AND BIOMATERIAL AND PRECURSOR AND PHARMACEUTICAL

=> s l1 and adhesion site
L2           0 L1 AND ADHESION SITE

=> growth factor
GROWTH IS NOT A RECOGNIZED COMMAND
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=> s l1 and growth factor
L3           8 L1 AND GROWTH FACTOR

=> s l3 and thiol and amine
L4           2 L3 AND THIOL AND AMINE

=> s l4 and half life
L5           2 L4 AND HALF LIFE

=> s l5 and pH
L6           2 L5 AND PH

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7           2 DUP REM L6 (0 DUPLICATES REMOVED)

=> d l7 bib abs 1-2
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L7 ANSWER 1 OF 2 USPATFULL
AN 2001:109883 USPATFULL
TI MULTI-ARMED, MONOFUNCTIONAL, AND HYDROLYTICALLY STABLE DERIVATIVES OF POLY (ETHYLENE GLYCOL) AND RELATED POLYMERS FOR MODIFICATION OF SURFACES AND MOLECULES
IN HARRIS, J. MILTON, HUNTSVILLE, AL, United States
VERONESE, FRANCESCO MARIA, PADOVA, Italy
CALICETI, PAOLO, PADOVA, Italy
SCHIAVON, ODDONE, PADOVA, Italy
PI US 2001007765 A1 20010712
AI US 1998-140907 A1 19980827 (9)
RLI Continuation of Ser. No. US 1995-443383, filed on 17 May 1995, GRANTED, Pat. No. US 5932462 Continuation-in-part of Ser. No. US 1995-371065, filed on 10 Jan 1995, ABANDONED
DT Utility
FS APPLICATION
LREP ALSTON & BIRD LLP, BANK OF AMERICA PLAZA, 101 SOUTH TRYON STREET, SUITE 4000, CHARLOTTE, NC, 28280-4000
CLMN Number of Claims: 67
ECL Exemplary Claim: 1
DRWN 8 Drawing Page(s)
LN.CNT 1800
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Multi-armed, monofunctional, and hydrolytically stable polymers are described having the structure ##STR1##

wherein Z is a moiety that can be activated for attachment to biologically active molecules such as proteins and wherein P and Q represent linkage fragments that join polymer arms poly.sub.a and poly.sub.b, respectively, to central carbon atom, C, by hydrolytically stable linkages in the absence of aromatic rings in the linkage fragments. R typically is hydrogen or methyl, but can be a linkage fragment that includes another polymer arm. A specific example is an mPEG disubstituted lysine having the structure ##STR2##

where mPEG.sub.a and mPEG.sub.b have the structure CH.sub.3O--(CH.sub.2CH.sub.2O).sub.nCH.sub.2CH.sub.2-- wherein n may be the same or different for poly.sub.a- and poly.sub.b- and can be from 1 to about 1,150 to provide molecular weights of from about 100 to 100,000.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 2 OF 2 USPATFULL
AN 1999:89039 USPATFULL
TI Multiarmed, monofunctional, polymer for coupling to molecules and surfaces
IN Harris, J. Milton, Huntsville, AL, United States
Veronese, Francesco Maria, Padua, Italy
Caliceti, Paolo, Padua, Italy
Schiavon, Oddone, Padua, Italy
PA Shearwater Polymers, Inc., Huntsville, AL, United States (U.S. corporation)
PI US 5932462 19990803
AI US 1995-443383 19950517 (8)
RLI Continuation-in-part of Ser. No. US 1995-371065, filed on 10 Jan 1995, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Naff, David M.
LREP Bell Seltzer Intellectual Property Law Group of Alston & Bird LLP
CLMN Number of Claims: 49
ECL Exemplary Claim: 1
DRWN 10 Drawing Figure(s); 8 Drawing Page(s)
LN.CNT 1683

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Multi-armed, monofunctional, and hydrolytically stable polymers are described having the structure ##STR1## wherein Z is a moiety that can be activated for attachment to biologically active molecules such as proteins and wherein P and Q represent linkage fragments that join polymer arms poly.sub.a and poly.sub.b, respectively, to central carbon atom, C, by hydrolytically stable linkages in the absence of aromatic rings and ester groups in the linkage fragments. R typically is hydrogen or methyl, but can be a linkage fragment that includes another polymer arm. A specific example is an mPEG disubstituted lysine having the structure ##STR2## where mPEG.sub.a and mPEG.sub.b have the structure CH.sub.3 O--(CH.sub.2 CH.sub.2 O).sub.n CH.sub.2 CH.sub.2 -- wherein n may be the same or different for mPEG.sub.a and mPEG.sub.b and can be from 1 to about 1,150 to provide molecular weights of from about 100 to 100,000. The mPEG disubstituted lysine can be purified from a reaction mixture by chromatography in water, including gel filtration chromatography and ion exchange chromatography because the carboxyl group is ionizable. Impurities are removed, including unreacted mPEG and mPEG monosubstituted lysine, to provide the polymer in pure form. Ion exchange chromatography permits fractionation of a greater amount of polymer per run.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l7 2 kwic

L7 ANSWER 2 OF 2 USPATFULL

SUMM . . . epsilon position as well as in the alpha position. The epsilon --NH.sub.2 is free for reaction under conditions of basic **pH**. Much of the art has been directed to developing polymer derivatives having active moieties for attachment to the epsilon --NH.sub.2. . .

SUMM The pendant carboxyl groups typically have been used to couple nonprotein **pharmaceutical** agents to the polymer. Protein **pharmaceutical** agents would tend to be cross linked by the multifunctional polymer with loss of protein activity.

SUMM . . . nonreactive end moiety so that the PEG molecule is monofunctional. Monofunctional PEGs are usually preferred for protein modification to avoid **cross linking** and loss of activity. One hydroxyl moiety on the terminus of the PEG diol molecule typically is substituted with a. . .

SUMM . . . hydrolysis can be avoided. Large linker fragments can be avoided so as to avoid an antigenic response in living organisms. **Cross linking** is avoided.

SUMM . . . mPEG moieties in the method for synthesizing the multi-armed structure. The linker fragments typically are alkyl fragments containing amino or **thiol** residues forming a linkage with the residue of the functional moiety of the polymer. Depending on the degree of substitution. . .

DRWD FIGS. 2(a) and 2(b) illustrate stability toward heat (FIG. 2(a)) and **pH** (FIG. 2(b)) of ribonuclease (.circle-solid.), linear mPEG-modified ribonuclease (.largecircle.), and ribonuclease modified with a multi-armed mPEG of the invention (.quadrature.).. . . incubation period at the indicated temperatures. FIG. 2(b) is based on data taken over a 20 hour period at different **pH** values.

DRWD . . . catalase (.quadrature.), and catalase modified with a multi-armed mPEG of the invention (.largecircle.) for 20 hours incubation at the indicated **pH** values.

DETD . . . organism or in a substance taken from a living organism. For example, an enzyme can catalyze chemical reactions. The term " **biomaterial**" is somewhat imprecise, and is used herein to refer to a solid material or particle or surface that is compatible. . .

DETD . . . mPEG-monosubstituted lysine, 353 milligrams of lysine, which is about 2.5 millimoles, was dissolved in 20 milliliters of water at a

pH of about 8.0 to 8.3. Five grams of mPEG-p-nitrophenylcarbonate of molecular weight 5,000, which is about 1 millimole, was added in portions over 3 hours. The **pH** was maintained at 8.3 with 0.2N NaOH. The reaction mixture was stirred overnight at room temperature. Thereafter, the reaction mixture was cooled to 0.degree. C. and brought to a **pH** of about 3 with 2N HCl. Impurities were extracted with diethyl ether. The mPEG monosubstituted lysine, having the mPEG substituted. . .

DETD . . . millimoles. The mixture of TEA and mPEG-monosubstituted lysine was dissolved in 10 milliliters of anhydrous methylene chloride to reach a **pH** of 8.0. Four and nine tenths grams of mPEG-p-nitrophenycarbonate of molecular weight 5,000, which is 1.056 millimoles, was added over. . . mPEG disubstituted compound having mPEG arms of different lengths, then a different molecular weight mPEG could have been used. The **pH** was maintained at 8.0 with TEA. The reaction mixture was refluxed for 72 hours, brought to room temperature, concentrated, filtered,. . . alkaline aqueous medium by stirring overnight at room temperature. The solution was cooled to 0.degree. C. and brought to a **pH** of about 3 with 2N HCl.

DETD . . . on a QAE Sephadex A50 column (Pharmacia) that measured 5 centimeters by 80 centimeters. An 8.3 mM borate buffer of **pH** 8.9 was used. This alternative procedure permitted fractionation of a greater amount of material per run than the other method. . .

DETD . . . at 0.degree. C. were 20 milliliters of anhydrous methylene chloride and 0.4 g of p-nitrophenylchloroformate, which is 2 millimoles. The **pH** of the reaction mixture was maintained at 8 by adding 0.28 milliliters of triethylamine ("TEA"), which is 2 millimoles. The. . .

DETD . . . is 5.4.times.10.sup.-4 moles, to 40 milliliters of lysine HCl solution. The lysine HCL solution was in a borate buffer of **pH** 8.0. The concentration was 0.826 milligrams succinimidylcarbonate mPEG per milliliter of lysine HCL solution, which is 1.76.times.10.sup.-4 moles. Twenty milliliters of the same buffer was added. The solution **pH** was maintained at 8.0 with aqueous NaOH solution for the following 8 hours. The reaction mixture was stirred at room. . .

DETD Thereafter, the solution was diluted with 300 milliliters of deionized water. The **pH** of the solution was adjusted to 3.0 by the addition of oxalic acid. The solution was then extracted three times. . .

DETD . . . is 500 milliliters of gel equilibrated with 1500 milliliters of boric acid in a 0.5% sodium hydroxide buffer at a **pH** of 7.0. The loaded system was then washed with water. Impurities of succinimidylcarbonate mPEG and mPEG-monosubstituted lysine, both of molecular. . . off the column. However, the desired mPEG disubstituted lysine of molecular weight 20,000 was eluted with 10 mM NaCl. The **pH** of the eluate was adjusted to 3.0 with oxalic acid and then mPEG disubstituted lysine was extracted with dichloromethane, dried. . .

DETD For the mPEG disubstituted lysines described above, --P--CR(--Q--)--Z is the reaction product of a **precursor** linker moiety having two reactive amino groups and active monofunctional precursors of poly.sub.a and poly.sub.b that have been joined to. . .

DETD . . . a wide variety of linker fragments P and Q are available, although not necessarily with equivalent results, depending on the **precursor** linker moiety and the functional moiety with which the activated mPEG or other nonpeptidic monofunctional polymer is substituted and from. . . result. Typically, the linker fragments will contain the reaction products of portions of linker moieties that have reactive amino and/or **thiol** moieties and suitably activated nonpeptidic, monofunctional, water soluble polymers.

DETD . . . wide variety of hydrolytically stable linkages with reactive amino moieties. Linkages can be selected from the group consisting of amide, **amine**, ether, carbamate, which are also called urethane linkages, urea, thiourea, thiocarbamate, thiocarbonate, thioether,

thioester, dithiocarbonate linkages, and others. However, hydrolytically. . .

DETD . . . of at least several days, and potentially indefinitely. Most proteins could be expected to lose their activity at a caustic **pH** of 11 or higher, so the derivatives should be stable at a **pH** of less than about 11.

DETD One or both of the reactive amino moieties, --NH.sub.2, of lysine or another linker moiety can be replaced with **thiol** moieties, --SH. Where the linker moiety has a reactive **thiol** moiety instead of an amino moiety, then the linkages can be selected from the group consisting of thioester, thiocarbonate, thiocarbamate,. . . The above linkages and their formation from activated mPEG and lysine in which both amino moieties have been replaced with **thiol** moieties are represented structurally below. ##STR21##

DETD . . . such as those shown above on the mPEG molecule to form hydrolytically stable linkages as discussed above. For example, the **amine** linkage could be formed as follows: ##STR22##

DETD Nucleophilic moieties for forming the linkages can be amino, **thiol**, and hydroxyl. Hydroxyl moieties form hydrolytically stable linkages with isocyanate electrophilic moieties. Also, it should be recognized that the linker. . .

DETD Linker moieties can be synthesized to include multiple reactive sites such as amino, **thiol**, or hydroxyl groups for joining multiple suitably activated mPEGs or other nonpeptidic polymers to the molecule by hydrolytically stable linkages,. . .

DETD . . . by which the same polymers are attached to lysine as shown above. Similarly, the amino groups can be replaced with **thiol** or other active groups as discussed above. However, only one hydroxyl group, which is relatively nonreactive, should be present in. . .

DETD . . . hydroxyl moiety, or activated carboxyl or hydroxyl moiety. The carboxyl and hydroxyl moieties are somewhat nonreactive as compared to the **thiol**, amino, and other moieties discussed above. The carboxyl and hydroxyl moieties typically remain intact when the polymer arms are attached. . .

DETD R.sub.Z can also include the reaction product of one or more reactive moieties including reactive amino, **thiol**, or other moieties, and a suitably activated mPEG arm or related nonpeptidic polymer arm. In the latter event, R.sub.Z can. . .

DETD . . . active form and its attachment to the central carbon, but the activated reactive site and also the conjugation of the **precursor** activated site with another molecule, whether that molecule be an enzyme, other protein or polypeptide, a phospholipid, a preformed liposome,. . .

DETD . . . methyl or other alkyl group, or can be the reaction product of one or more reactive moieties including reactive amino, **thiol**, or other moieties, and a suitably activated mPEG arm or related nonpeptidic polymer arm. In the latter event, R can. . .

DETD . . . located at least one active site for which the monofunctional, nonpeptidic polymers are selective. These active sites include amino moieties, **thiol** moieties, and other moieties as described above.

DETD A highly useful, new activating group that can be used for highly selective coupling with **thiol** moieties instead of amino moieties on molecules and surfaces is the vinyl sulfone moiety described in U.S. Pat. No. 5,446,090,. . . herein by reference. Various sulfone moieties can be used to activate a multi-armed structure in accordance with the invention for **thiol** selective coupling.

DETD . . . of the two-armed mPEG were used. However, in all examples the enzymes were dissolved in a 0.2M borate buffer of **pH** 8.5 to dissolve proteins. The polymers were added in small portions for about 10 minutes and stirred for over 1. . .

DETD . . . gel filtration chromatography on a Pharmacia Superose 12 column, operated by an FPLC instrument, using 10 mM phosphate buffer of **pH** 7.2, 0.15M in NaCl, as eluent.

DETD . . . dehydrogenase, 100 .mu.g of NADH, 0.5 .mu.g of asparaginase and 10 .mu.moles of asparagine were incubated in 0.122M Tris buffer, **pH** 8.35, while the NADH absorbance decrease at 340 nm was followed.

DETD Proteolytic digestion was performed in 0.05M phosphate buffer of **pH** 7.0. The free enzyme, linear mPEG and protein conjugate, and two-armed mPEG-protein conjugates were exposed to the known proteolytic enzymes. . .

DETD D. Blood Clearance Times. Increased blood circulation half lives are of enormous **pharmaceutical** importance. The degree to which mPEG conjugation of proteins reduces kidney clearance of proteins from the blood was determined and. . .

DETD For linear mPEG-uricase and two-armed mPEG-uricase, with 40% modification of lysine groups, the **half life** for blood clearance was 200 and 350 minutes, respectively. For unmodified uricase the result was 50 minutes.

DETD . . . of native ribonuclease, catalase and asparaginase and their linear mPEG and two-armed mPEG conjugates was evaluated in 0.5M phosphate buffer **pH** 7.0 at 1 mg/ml, 9 .mu.g/ml and 0.2 mg/ml respectively. The samples were incubated at the specified temperatures for 15. . .

DETD Increased thermostability was found for the modified forms of ribonuclease, as shown in FIG. 2, at **pH** 7.0, after 15 min. incubation at different temperatures, but no significant difference between the two polymers was observed. Data for. . .

DETD F. **pH** Stability of the Free and Conjugated Enzymes. Unmodified and polymer-modified enzymes were incubated for 20 hrs in the following buffers: sodium acetate 0.05M at a **pH** of from 4.0 to 6.0, sodium phosphate 0.05M at **pH** 7.0 and sodium borate 0.05M at a **pH** of from 8.0 to 11. The enzyme concentrations were 1 mg/ml, 9 .mu.g/ml, 5 .mu.g/ml for ribonuclease, catalase, and asparaginase respectively. The stability to incubation at various **pH** was evaluated on the basis of enzyme activity.

DETD As shown in FIG. 2b, a decrease in **pH** stability at acid and alkline **pH** values was found for the linear and two-armed mPEG-modified ribonuclease forms as compared to the native enzyme. As shown in FIG. 4, stability of the linear mPEG and two-armed mPEG conjugates with catalase was improved for incubation at low **pH** as compared to native catalase. However, the two-armed mPEG and linear mPEG conjugates showed equivalent **pH** stability. A limited increase in **pH** stability at acid and alkaline **pH** values was noted for linear and two-armed mPEG-modified asparaginase as compared to the native enzyme.

DETD . . . P, dynorphin, oxytocin and growth hormone-releasing peptide, tumor necrosis factor binding protein, growth factors such as growth hormone (GH), insulin-like **growth factor** (IGF-I, IGF-II), .beta.-nerve **growth factor** (.beta.-NGF), basic fibroblast **growth factor** (bFGF), transforming **growth factor**, erythropoietin, granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), platelet-derived **growth factor** (PDGF) and epidermal **growth factor** (EGF), enzymes such as tissue plasminogen activator (t-PA), elastase, superoxide dismutase (SOD), bilirubin oxydase, catalase, uricase and asparaginase, other proteins. . .

CLM What is claimed is:

. . . polymer of claim 1 wherein said linkage fragments P and Q comprise moieties selected from the group consisting of amide, **amine**, ether, carbamate, thiourea, urea, thiocarbamate, thiocarbonate, thioether, thioester, and dithiocarbamate moieties.

13. The polymer of claim 3 wherein said nucleophilic moieties are selected from the group consisting of amino, **thiol**, and hydroxyl moieties.

. . . method of claim 25 wherein the active moiety W is a nucleophilic moiety selected from the group consisting of amino, **thiol**, and hydroxyl moieties.

35. The method of claim 25 wherein the linkages are selected from the group consisting of amide, **amine**, ether, carbamate, thiourea, urea, thiocarbamate, thiocarbonate, thioether, thioester, and dithiocarbamate linkages.

. . . with nucleophilic moieties is an electrophilic moiety that is reactive with nucleophilic moieties selected from the group consisting of amino, **thiol**, and hydroxyl moieties.

40. The method of claim 39 wherein the reaction takes place in water at a **pH** of about 8.0.

=>